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Dated 17 · 5 · 1996

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Corporate name

Country *(and State of incorporation, if appropriate)*

2e If you are applying as an individual or one of a partnership please give in full:

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3a Have you appointed an agent to deal with your application?

Yes [X] No [ ] ➡ ***go to 3b***

↓ ***please give details below***

Agent's name: J. MILLER & CO.

Agent's address: 34 BEDFORD ROW
HOLBORN
LONDON

Postcode: WC1R 4JH

Agent's ADP number: 1149002

3b: *If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.*

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21 AUG 1995

23AUG95 E136539-1 000528
P01/7700 25.00

Reference GBP11706A

9517107.0

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The Patent Office

Request for grant of a Patent

Form 1/77

Patents Act 1977

❶ Title of invention

1 Please give the title of the invention: FATTY ACID ESTERS

❷ Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate name: SCOTIA HOLDINGS PLC

Country *(and State of incorporation, if appropriate)*: ENGLAND

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Country: ENGLAND

ADP number *(if known)*: 6726021800 1

The answer must be 'No' if:
- *any applicant is not an inventor*
- *there is an inventor who is not an applicant, **or***
- *any applicant is a corporate body.*

Inventorship

7 Are you (the applicant or applicants) the sole inventor or the joint inventors?

Please mark correct box

Yes ☐ No ☒ ➡ ***A Statement of Inventorship on Patents Form 7/77 will need to be filed (see Rule 15).***

Please supply duplicates of claim(s), abstract, description and drawing(s).

Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77			
Claim(s)		Description	13
Abstract		Drawing(s)	

8b Which of the following documents also accompanies the application?

Please mark correct box(es)

Priority documents *(please state how many)*	
Translation(s) of Priority documents *(please state how many)*	
Patents Form 7/77 – Statement of Inventorship and Right to Grant *(please state how many)*	
Patents Form 9/77 – Preliminary Examination/Search	
Patents Form 10/77 – Request for Substantive Examination	

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Request

I/We request the grant of a patent on the basis of this application.

Please sign here ➡

Signed J. Miller & Co. Date 21 August 1995 *(day month year)*

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❶ Reference number

4 Agent's or applicant's reference number *(if applicable)* GBP11706A

❺ Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ ➡ ***go to 6***

⬇ ***please give details below***

❑ number of earlier application or patent number

❑ filing date

(day month year)

❑ and the Section of the Patents Act 1977 under which you are claiming:

Please mark correct box

15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

❻ Declaration of priority

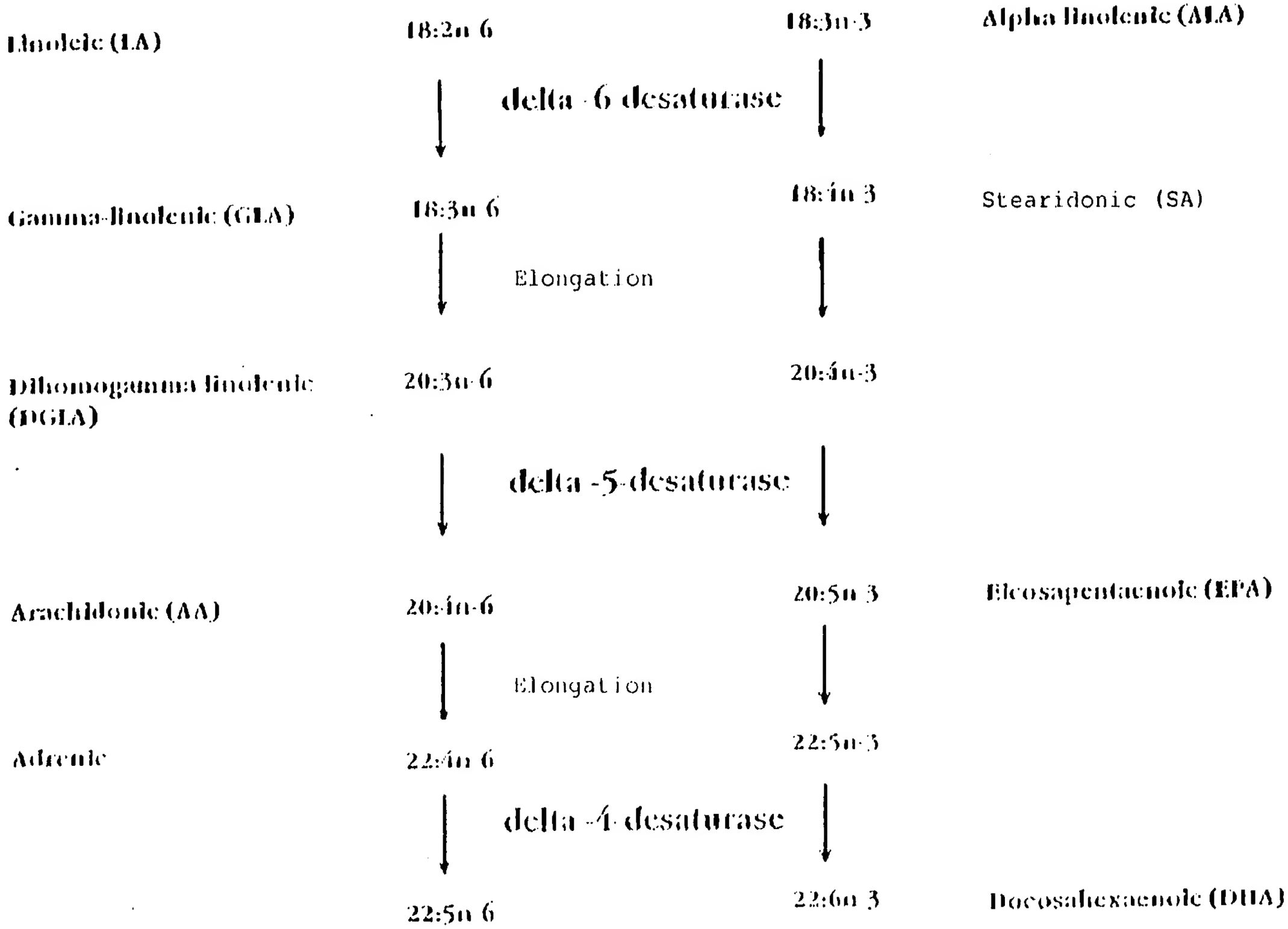
❻ *If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number.*

Please give the date in all number format, for example, 31/05/90 for 31 May 1990.

6 If you are declaring priority from previous application(s), please give:

Country of filing	Priority application number *(if known)*	Filing date *(day, month, year)*

Scheme 1 :


Linoleic (LA)
18:2n 6
18:3n-3
Alpha linolenic (ALA)
delta -6 desaturase
Gamma-linolenic (GLA)
18:3n 6
18:4n 3
Stearidonic (SA)
Elongation
Dihomogamma linolenic (DGLA)
20:3n-6
20:4n-3
delta -5-desaturase
Arachidonic (AA)
20:4n-6
20:5n 3
Eicosapentaenoic (EPA)
Elongation
Adrenic
22:4n 6
22:5n-3
delta -4-desaturase
22:5n 6
22:6n 3
Docosahexaenoic (DHA)

FATTY ACID ESTERS

Field of Invention

This invention relates to fatty acid esters.

Essential Fatty Acids

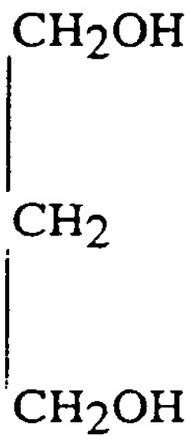
The essential fatty acids (EFAs) consist of a series of twelve compounds illustrated in Scheme 1. Although linoleic acid, the parent compound of the n-6 series, and alpha-linolenic acid, the parent compound of the n-3 series, are the main dietary EFAs, these substances as such have relatively minor roles in the body. In order to be fully useful to the body, the parent compounds must be metabolised by the sequence of reactions shown in Scheme 1. In quantitative terms, as judged by their levels in cell membranes and in other lipid fractions, dihomogammalinolenic acid (DGLA) and arachidonic acid (AA) are the main EFA metabolites of the n-6 series while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main metabolites of the n-3 series. DGLA, AA, EPA and DHA are important constituents of most of the lipids in the body. As well as being important in themselves they can also give rise to a wide range of oxygenated derivatives, the eicosanoids, including the prostaglandins, leukotrienes and other compounds.

The conversions of the essential fatty acids are irreversible in the human body and are achieved by successive desaturation and elongation steps as shown in Scheme 1.

The Invention

To date, proposals have been in terms of particular triglycerides, following the natural occurrence of essential fatty acids in triglyceride form. However, triglycerides, unless symmetrical about the 2-carbon, are chiral and that fact, coupled with acyl migration between the terminal and 2-carbon positions, makes the synthesis of specific triglycerides a difficult task.

We have seen that for purposes of convenient administration of different fatty acids simultaneously, or indeed of a single fatty acid in high amounts in well tolerated form, use can be made of esters of 1, 3-propane diol:-



and related compounds of longer chain length or with one or both of the hydroxyl groups not terminal; 1,3-propane diol being however preferred as well tolerated in the body, similar to glycerol itself.

Fatty acid diesters of said diols are believed to be new in themselves, certainly when of biologically significant fatty acids and in particular when of the n-6 and n-3 EFAs or related long chain unsaturated acids including oleic acid or other more unusual acids such as parinaric acid and columbinic acid. However, the invention specifically extends to the diesters when for use in therapy or nutrition generally, and when for use in preparation of medicaments for particular therapeutic purposes, including therapy of the conditions specifically mentioned herein.

Variations of the Invention

A number of variants of the diesters may be summarised as classes 1 to 6 below, in which possible variations of the diol are explored, together with use of fatty acid alcohols instead of, or in part instead of, the fatty acids.

The elongation reactions shown in Scheme 1, in which two carbon atoms are added to the chain, tend to be very rapid, whereas the desaturation reactions in which an extra double bond is introduced tend to be very slow. Thus gamma-linolenic acid (GLA) is rapidly converted to DGLA, and stearidonic acid (18:4n-3) is readily converted to 20:4n-3; these pairs of compounds are biologically equivalent. DGLA is, however, for example, only slowly converted to AA.

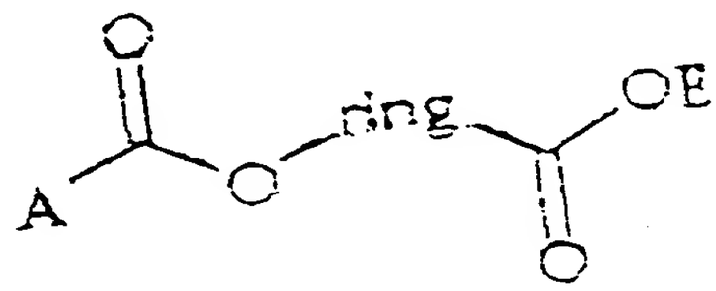
It has become increasingly apparent that in many different disease states there are abnormalities of EFA biochemistry leading to abnormal EFA levels in various lipid fractions in various tissues. These diseases include diseases of the heart and circulation such as hypertension and coronary and peripheral vascular disease, diseases of inflammation and immunity such as atopic disorders, osteoarthritis, rheumatoid arthritis, ulcerative colitis, Crohn's disease and various disorders going under the general classifications of inflammatory or auto-immune, neurological disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis, disorders of the kidney, disorders of the skin, disorders of the gastrointestinal tract, disorders of calcium and other minerals, disorders of bone and connective tissue, disorders of the reproductive and endocrine systems, psychiatric disorders including schizophrenia, and disorders of ageing.

It used to be thought that, both in nutrition and in therapy of disease, it was sufficient to supply linoleic and alpha-linolenic acids and the body's own metabolism would do the rest. It is now evident that this is not true. Different diseases may have different abnormal patterns of EFAs and because of problems in metabolism these cannot simply be corrected by giving linoleic or alpha-linolenic acid. It may therefore be appropriate in some situations to give two or more of the EFAs simultaneously. While the EFAs can be supplied in various forms and in various mixtures, it would be convenient in both nutrition and in medical treatment to be able to supply the fatty acids as particular molecules.

E = the carbon chain of a fatty alcohol produced by reduction of any fatty acid, especially oleic acid and those illustrated in Scheme 1;

chain = any chain (either fully hydrocarbon in nature or containing heteroatom(s)) especially $(CH_2)_n$ where n = 1 to 10.

Class 4

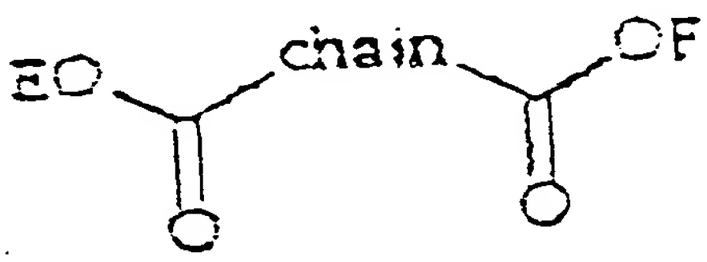


A = as before;

E = as before;

ring = any ring, either carbocyclic or heterocyclic, saturated or unsaturated, especially such that the final compound is non-chiral.

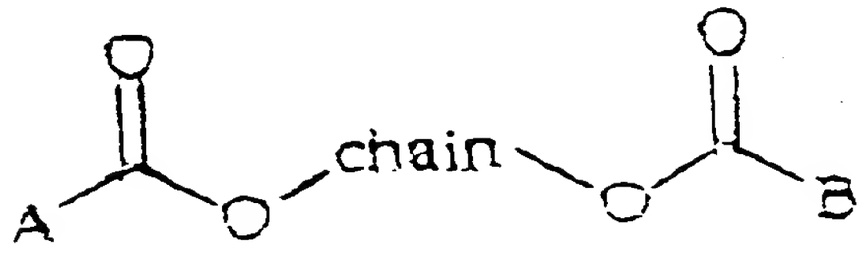
Class 5



E,F = as for E before the same or different.

chain = any chain (either fully hydrocarbon in nature or containing heteroatom(s)) especially $(CH_2)_n$ where n = 1 to 10.

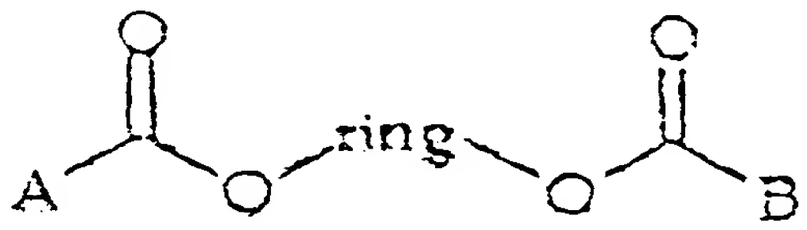
Class 1



A,B = any fatty acid carbon chain, the same or different, especially when derived from oleic acid and the acids illustrated in Scheme 1;

chain = any chain (either fully hydrocarbon in nature or containing heteroatom(s)) especially $(CH_2)_n$ where n=1 to 10.

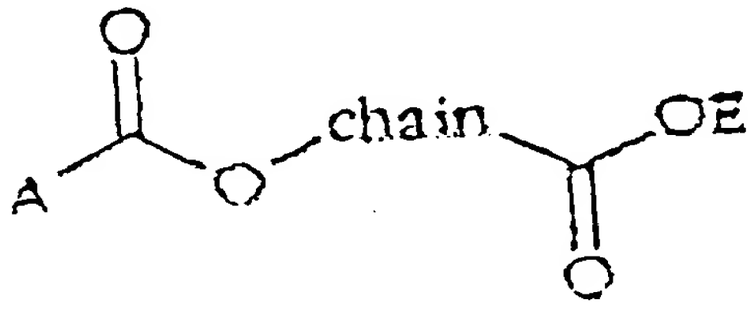
Class 2



A,B = as before;

ring = any ring, either carbocyclic or heterocyclic, saturated or unsaturated, especially such that the final compound is non-chiral.

Class 3



A = as before;

pyridine, in a suitable inert solvent, e.g. methylene chloride, and at a temperature between 0°C and 120°C.

(ii) by reaction of the diol with an acid or a short or medium chain length ester c the acid, in the presence of a suitable acid catalyst, e.g. p-toluenesulphonic acid, with or without a suitable inert solvent, e.g. toluene, at a temperature between 50° C and 180°C such that the water or alcohol formed in the reaction is removed by azeotropy or under vacuum.

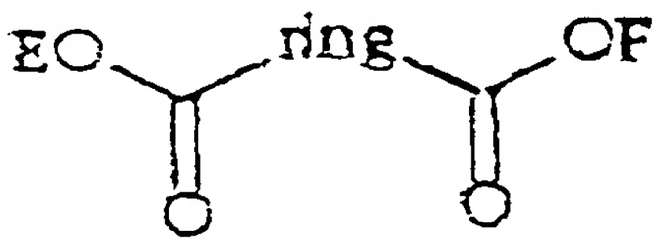
(iii) by reaction of the diol with an acid in the presence of a condensing agent, e.g. 1,3-dicyclohexylcarbodiimide with or without a suitable base, e.g. 4-(N,N-dimethylamino)pyridine, in an inert solvent, e.g. methylene chloride, at a temperature between 0°C and 50°C.

(iv) by reaction of the diol with an acid or a short or medium chain length ester of the acid, or an activated ester thereof, e.g. vinyl, trifluoroethyl, in the presence of a hydrolase enzyme with or without a suitable solvent, e.g. hexane at temperatures between 20°C and 80°C under conditions such that the water or alcohol by-product formed in the reaction is removed from the reaction mixture, e.g. molecular sieves, vacuum.

(v) by reaction of an acid with a suitable diol derivative, e.g. tosylate, iodide, with or without the presence of a suitable base, e.g. potassium carbonate, in a suitable inert solvent, e.g. dimethylformamide, and at a temperature between 0°C and 180°C.

(vi) by reaction of an acid ester (acid-CO_2Y) with the diol in the presence of a catalytic amount of an alkoxide of type M^+OY^- where M is an alkali or alkaline earth metal, e.g. sodium, and Y is an alkyl group containing 1-4 carbon atoms which may be branched, unbranched, saturated or unsaturated. The reaction is carried out with or without a suitable solvent, e.g. toluene, at temperatures between 50°C and 180°C such that the lower alcohol, HOY, is removed from the reaction mixture, e.g. by azeotropy or vacuum.

Class 6



E,F = as before

ring = any ring, either carbocyclic or heterocyclic, saturated or unsaturated, especially such that the final compound is non-chiral.

Syntheses

As far as we are aware, all of the compounds specified are new entities which do not appear in nature and have not previously been described. They may be prepared as follows:-

1. The individual fatty acids may be purified from natural animal, vegetable or microbial sources or may be chemically synthesised by methods well known to those skilled in the art or by methods to be developed in the future.

2. The individual fatty alcohols may be prepared by chemical reduction of the fatty acids outlined in 1 above by methods well known to those skilled in the art or by methods to be developed in the future.

3. The diesters may be prepared by a range of ester forming reactions well known to those skilled in the art or by methods to be developed in the future and especially:-

(i) by reaction of the diol with an acid chloride, acid anhydride or other suitable acid derivative with or without the presence of an organic tertiary base, e.g.

Example 2

1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-(z-octadec-9-enoyloxy)propane. (Class 1)

Part 1: 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane

A solution of z,z,z-octadeca-6,9,12-trienoic acid (150 parts, g) in methylene chloride (500 parts, ml) was added dropwise to a mixture of 1,3-dihydroxypropane (205 parts, g), 1,3-dicyclohexylcarbodiimide (130 parts, g) and 4-(N,N-dimethylamino) pyridine (87 parts, g) in methylene chloride (2500 parts, ml) at room temperature under nitrogen. When tlc indicated that the reaction had gone to completion, the reaction mixture was filtered. The filtrate was washed with dilute hydrochloric acid, water and saturated sodium chloride solution. The solution was dried, concentrated and purified by dry column chromatography to yield 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane as a pale yellow oil.

Part 2 : 1-(z,z,z,-octadeca-6,9,12-trienoyloxy)-3-(z-octadec-9-enoyloxy)propane

A solution of 1,3-dicyclohexylcarbodiimide (23.7 parts, g) and 4-(N,N-dimethylamino) pyridine (15.9 parts, g) in methylene chloride (200 parts, ml) was added to a solution of 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane (33.6 parts, g) and z-octadec-9-enoic acid (30 parts, g) in methylene chloride (400 parts, ml) under nitrogen at room temperature. On completion of reaction as evidenced by tlc analysis, the solution was diluted with hexane, filtered, concentrated and purified by dry column chromatography to yield 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-(z-octadec-9-enoyloxy) propane as a free flowing pale yellow oil.

Example 3

1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)propane. (Class 1)

Prepared as in Example 2, Part 2 but replacing z-octadeca-9-enoic acid with z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic acid. Chromatography yielded 1-(z,z,z-

Uses

These EFA and other fatty acid diesters have a wide variety of possible uses. They may be used as pharmaceuticals for the treatment or prevention of diseases in which abnormalities of EFAs have been identified. They may be added to foods or added to or used as nutritional supplements for those who require the particular EFAs for the treatment or prevention of diseases. They may also be used in foods or pharmaceuticals for veterinary use. They may also be used for skin care.

The diesters may be formulated in any way appropriate and which is known to those skilled in the art of preparing pharmaceuticals, skin care products or foods. They may be administered orally, enterally, topically, parenterally (subcutaneously, intramuscularly, intravenously or by any other route), rectally, vaginally or by any other appropriate route.

The doses to be administered range from 1 mg to 50 g per day, preferably 10 mg to 10 g and very preferably 10 mg to 2 g. They may be administered topically in preparations containing from 0.001% to 50% of the topical preparation, preferably 0.05% to 20% and very preferably 0.1% to 10%.

Preparative Examples

Example 1

1,3-(di-z,z,z-octadeca-6,9,12-trienoyloxy)propane. (Class 1)

A solution of 1,3-dicyclohexylcarbodiimide (1.07 parts, g) and 4-(N,N-dimethylamino) pyridine (0.59 parts, g) in methylene chloride (5 parts, ml) was added to a solution of 1,3-dihydroxypropane (0.152 parts, ml) and z,z,z-octadeca-6,9,12-trienoic acid (95%, 1.36 parts, g) in methylene chloride (15 parts, ml). The reaction was stirred at room temperature under nitrogen until it was complete as determined by tlc. Hexane (80 parts, ml) was added to the reaction. The precipitate was removed by filtration and washed thoroughly with hexane. The combined filtrates were concentrated and purified by flash chromatography to yield 1,3-(di-z,z,z-octadeca-6,9,12-trienoyloxy)propane as a pale yellow free flowing oil.

Example 6

1-(z,z,z-octadeca-6,9,12-trienoyloxy)-4-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)benzene. (Class 2)

Prepared as in Example 5, Parts 1 and 2 but replacing 1,5-dihydroxypentane with 1,4-dihydroxybenzene in Part 1 and replacing methylene chloride with tetrahydrofuran as the solvent in Part 1. Chromatography yielded 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-4-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)benzene as a pale yellow oil.

Example 7

z,z,z-octadeca-6,9,12-trienyl-z,z,z-octadeca-6,9,12-trienoate. (Class 3)

1,3-dicyclohexylcarbodiimide (0.82 parts, g) and 4-(N,N-dimethylamino)pyridine (0.48 parts, g) in methylene chloride (5 parts, ml) were added to a solution of z,z,z-octadeca-6,9,12-trienol (0.95 parts, g) and z,z,z-octadeca-6,9,12-trienoic acid (1 part, g) in methylene chloride (10 parts, ml) with stirring at room temperature under nitrogen. On completion of reaction as evidenced by tlc, hexane was added to the reaction mixture which was subsequently filtered and purified by column chromatography to yield z,z,z-octadeca-6,9,12-trienyl-z,z,z-octadeca-6,9,12-trienoate as a pale yellow oil.

Example 8

z,z,z-octadeca-6,9,12-trienyl-z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoate. (Class 3)

Prepared as in Example 8 but replacing z,z,z-octadeca-6,9,12-trienoic acid with z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic acid.

Example 9

1,4-di(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate. (Class 5)

Part 1:1-(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate.

A solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (0.54 parts, ml) in dry tetrahydrofuran (10 parts, ml) was added dropwise to a cooled (0°C) solution of z,z,z-octadeca-6,9,12-trienol (1 part, g) and succinic anhydride (0.36 parts, g) in dry

octadeca-6,9,12-trienoyloxy)-3-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)propane as a pale yellow oil.

Example 4

1,3-di(z,z,z-octadeca-6,9,12-trienoyloxy)propane. (Class 1)

Prepared as in Example 2, Part 2 but replacing z-octadeca-9-enoic acid with z,z,z-octadeca-6,9,12-trienoic acid. Chromatography yielded 1,3-di(z,z,z-octadeca-6,9,12-trienoyloxy)propane as a pale yellow oil.

Example 5

1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)pentane. (Class 1)

Part 1: 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-hydroxypentane.

z,z,z-Octadeca-6,9,12-trienoyl chloride (2 parts, g) was added dropwise to a solution of 1,5-dihydroxypentane (3.5 parts, g), triethylamine (0.94 parts, ml) and 4-(N,N-dimethylamino) pyridine (0.2 parts, g) in methylene chloride (50 parts, ml) with stirring at 0°C under nitrogen. On completion of reaction as evidenced by tlc the reaction mixture was washed with dilute hydrochloric acid and water, dried and purified by column chromatography yielding 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-hydroxypentane as a pale yellow oil.

Part 2: 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)pentane.

As for Example 2, Part 2 but replacing 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane with 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-hydroxypentane and z-octadeca-9-enoic acid with z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic acid. Chromatography yielded 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)pentane as a pale yellow oil.

tetrahydrofuran (20 parts, ml). On completion of reaction as evidenced by tlc, the reaction mixture was diluted with diethyl ether and washed with dilute hydrochloric acid, water and brine. The organic layer was dried, concentrated and used directly in the second part of the reaction.

Part 2: 1,4-di(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate.

A solution of 1,3-dicyclohexylcarbodiimide (0.83 parts, g) and 4-(N,N-dimethylamino) pyridine (0.55 parts, g) in methylene chloride (20 parts, ml) was added to a solution of 1-(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate (1.32 parts, g) and z,z,z-octadeca-6,9,12-trienol (0.98 parts, g) in methylene chloride (40 parts, ml). On completion, as evidenced by tlc analysis, the reaction mixture was diluted with hexane, filtered, concentrated and purified by chromatography to yield 1,4-di(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate as a pale yellow oil.